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Resistance and susceptibility to powdery mildew, root-knot nematode, and western flower thrips in two types of winter cress (Brassicaceae)

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Abstract. Two types of *Barbarea vulgaris* R. Br. (Brassicaceae) were tested to compare their resistance and susceptibility to powdery mildew, *Erysiphe cruciferarum* Opiz ex L. Junell (Erysiphales: Erysiphaceae), root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwoodi (Tylenchida: Heteroderidae), and western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). In experiments conducted in the greenhouse, the percentage of plants showing powdery mildew symptoms ranged from 54.2 to 83.3% in P-type *B. vulgaris* plants and from 0 to 20.8% in G-type *B. vulgaris* plants. In plants infected by powdery mildew, the percentage of leaves affected was higher in P-type than in G-type plants, ranging from 11.8 to 21.1% in P-type plants, and from 0 to 0.36% in G-type plants. Infection by powdery mildew was more likely to occur on the leaves of largest diameter. Root galling showed that G- and P-type plants were equally attacked by root-knot nematode, but the multiplication rate of the nematode was 4.1 to 7.6 times higher in P-type than in G-type plants. Significantly more leaves per plant were damaged by western flower thrips in P-type (73.1 to 88.3% of leaves affected) than in G-type plants (2.1 to 2.9% of leaves affected). The total numbers of adult and immature thrips found per plant on P-type plants were, respectively, 29.9 and 2.5, while on G-type plants less than 0.3 adult and immature thrips were found per plant. This study indicates that G-type *B. vulgaris* could be a source of resistance to powdery mildew and western flower thrips.

Keywords: *Barbarea vulgaris*, *Erysiphe cruciferarum*, *Frankliniella occidentalis*, *Meloidogyne incognita*, host-plant resistance

1. Introduction

Barbarea vulgaris R. Br. (Brassicaceae), commonly known as winter cress and yellow rocket, is a biennial or short-lived perennial plant that occurs in temperate regions worldwide (MacDonald and Cavers, 1991). *Barbarea vulgaris* has been shown to have two morphologically-distinct forms, G and P, which have hairless (**G**labrous) and hairy (**P**ubescent) leaves, respectively, and which also differ in the content of glucosinolates and saponins, are genetically divergent, and show differences in habitat adaptation (Agerbirk et al., 2015; Agerbirk et al., 2003b; Christensen et al., 2014; Christensen et al., 2016; Hauser et al., 2012; Heimes et al., 2016; Toneatto et al., 2010). Henceforth we will refer to G-type *B. vulgaris* var. *arcuata* as G-type and to P-type *B. vulgaris* as P-type. The G-type contains the triterpenoid saponins 3-*O*-*β*-cellobiosylhederagenin and 3-*0*-*β*-cellobiosyloleanolic acid, which make this plant resistant to the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), and the flea beetle *Phyllotreta nemorum* L. (Coleoptera: Chrysomelidae) (Agerbirk et al., 2003a; Agerbirk et al., 2001; Augustin et al., 2012; Badenes-Pérez et al., 2014b; Badenes-Pérez et al., 2010; Idris and Grafius, 1994; Kuzina et al., 2009; Kuzina et al., 2011; Nielsen et al., 2010a; Nielsen et al., 2010b; Shelton and Nault, 2004; Shinoda et al., 2002). The P-type does not contain these saponins and allows the development and survival of *P. nemorum* and *P. xylostella* (Agerbirk et al., 2003a; Badenes-Pérez et al., 2010). However, the P-type is often resistant to the oomycete pathogen causing white rust, *Albugo candida* (Pers.) Kuntze (Peronosporales: Albuginaceae), while the G-type is mostly susceptible to it (Christensen et al., 2014; Heimes et al., 2014; van Mölken et al., 2014). Within *B. vulgaris*, two biochemically

distinct forms that are morphologically indistinguishable have also been found (Agerbirk et al., 2015; van Leur et al., 2006). BAR and NAS *B. vulgaris* plants have (*S*)-2-hydroxy-2-phenylethylglucosinolate (glucobarbarin) and 2-phenylethylglucosinolate (gluconasturtiin), respectively, as their main glucosinolates (Agerbirk et al., 2015; van Leur et al., 2006). The preference and performance of insects on BAR and NAS plants has been tested with *Delia radicum* L. (Diptera: Anthomyiidae), *Mamestra brassicae* L. (Lepidoptera: Noctuidae), *Pieris rapae* L. (Lepidoptera: Pieridae), and *P. xylostella* (van Leur et al., 2008a; van Leur et al., 2008b). Significant differences have been found in the performance of these insects, which grew better either on BAR plants (*D. radicum*) or on NAS plants (*M. brassicae*) (van Leur et al., 2008a; van Leur et al., 2008b), while NAS plants were preferred by ovipositing *P. xylostella* over BAR plants (Badenes-Pérez et al., 2014b).

The root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwoodi (Tylenchida: Heteroderidae), the ascomycete fungus causing powdery mildew on many Brassicaceae, *Erysiphe cruciferarum* Opiz ex L. Junell (Erysiphales: Erysiphaceae), and the western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) are important pests that cause significant economic damage in cruciferous crops (Jones et al., 2013; Reitz, 2009; Sasser, 1980; Uloth et al., 2016; Van de Wouw et al., 2016). Many different plant species have been tested as host-plants for *M. incognita* (Curto et al., 2005; Edwards and Ploeg, 2014), but not *B. vulgaris.* A different *Barbarea* sp., *B. verna* (Mill.) Asch has been considered a poor host-plant for *M. incognita* (Curto et al., 2005). The mildew *E. cruciferarum* is an emerging threat in the production of some *Brassica* species (Uloth et al., 2016). Moderate resistance and tolerance to *E. cruciferarum* has been

identified in some *Brassica* species and cultivars, but reports are few (Bradshaw et al., 1989; Singh et al., 2010; Uloth et al., 2016). The thrips *F. occidentalis* is polyphagous, but it has been shown to exhibit strong host plant preferences (Cao et al., 2014; Kirk and Terry, 2003; Nyasani et al., 2013). Reports of host-plant resistance to this thrips are also scarce (De Jager et al., 1995; Kos et al., 2014; Leiss et al., 2009; Maharijaya et al., 2012; Mirnezhad et al., 2010). Here we compare the differences in resistance and susceptibility to the mildew *E. cruciferarum*, the nematode *M. incognita*, and the thrips *F. occidentalis* in G- and P-type plants.

2. Materials and methods

Experiments to test the resistance and susceptibility of the different *Barbarea* plants to the mildew *E. cruciferarum* and the thrips *F. occidentalis* were conducted in the greenhouse at 25 ± 3 ºC at the Institute of Agricultural Sciences in Madrid, Spain. Experiments to test the resistance and susceptibility of G- and P-type plants to the nematode *M. incognita* were conducted at the Centro de Investigación Apícola y Agroambiental in Marchamalo, Spain. The experiments with the nematode *M. incognita* were conducted in semi-field conditions in a greenhouse-covered field at 9-43 ºC in 2012 and in a growth chamber in the laboratory at 24 ± 1 ºC in 2017. Seeds of G- and P-type plants were originally from Denmark. They were donated to us directly by Drs. Niels Agerbirk and Jens K. Nielsen in 2010 and 2014 (see accessions B44 and B4 for G-and P-type seeds, respectively, in Agerbirk et al. (2003a, b)). Additional seeds from Denmark were donated to us by the "César Gómez Campo" seed bank at the Universidad Politécnica de Madrid (accessions BGV-UPM 9971 and BGV-UPM 10053). These seeds from the "César Gómez

Campo" seed bank were deposited there by Dr. Niels Agerbirk and they were handed to us by Drs. Itziar Aguinagalde Madariaga, David Draper Munt, and M. Elena González-Benito in 2010 and 2012. The seeds provided by Dr. Niels Agerbirk were also used to grow plants with the purpose of multiplication, and seeds obtained from these plants were used in the experiment conducted with the nematode *M. incognita* in 2017. *Barbarea rupicola* Moris (B & T World Seeds, Aigues-Vives, France); *B. verna* (Mill.) Asch. (Johnny´s Selected Seeds, Albion, ME, USA); cauliflower, *Brassica oleracea* L. var. *botrytis*, cultivar 'Snowball' (Intersemillas, Quart de Poblet, Spain); Indian mustard, *Brassica juncea* (L.) Czern, cultivar 'Pacific Gold' (Johnny´s Selected Seeds, Albion, ME, USA); and tomato, *Solanum lycopersicum* L. cultivar 'Marmande' (Rocalba, Girona, Spain), were also used. Plants used in the experiments were grown in a peat moss substrate in the greenhouse in 15-cm pots and they were fertilized fortnightly with an all-purpose fertilizer (Nutrichem 60, Miller Chemical and Fertilizer Corp., Hanover, PA, USA). In the case of the plants used to test resistance and susceptibility to the nematode *M. incognita*, the substrate used consisted of 30% peat moss, 35% coarse sand, and 35% crumb sand (v/v).

2.1 Resistance and susceptibility to powdery mildew

A total of 24 plants of each type were used in each of three replications of an experiment conducted to test differences in resistance and susceptibility to the mildew *E. cruciferarum* in G- and P-type plants. The experiment relied on the *E. cruciferarum* that naturally occurred in the greenhouse where the experiment was conducted. Plants of the G- and P-types were randomly located on a bench at the greenhouse and the minimum distance between adjacent plants was 16 cm. In the experiment the percentage of leaves

per plant showing symptoms of *E. cruciferarum* infection visible with the naked eye were recorded. In the first replication of the experiment, only G- and P-type plants were used and plants were three months old when the observations were made in December 2014. In this experiment we also recorded total number of leaves per plant, leaf diameter, and whether symptoms of *E. cruciferarum* infection were present in five plants of each type. To record leaf diameter per plant, the plant was approximately divided in half (two sections), one of the sections was randomly chosen, and the leaf diameters of all leaves in the section were recorded. In the second and third replications of the experiment the plants used were 10 months old when the observations were made in September 2015 and December 2015. In the second and third replications of the experiment, besides G- and P-type plants, *B. rupicola* and *B. verna* plants were also included.

2.2 Resistance and susceptibility to root-knot nematode

The population of the nematode *M. incognita* used in the experiment was isolated from highly infested roots of cucumber plants grown in a greenhouse in Marchamalo, Spain. Females were identified as *M. incognita* according to their isoesterase electrophoretic pattern (Esbenshade and Triantaphyllou, 1990). To prepare the inoculum of *M. incognita* nematodes, eggs were extracted by macerating the roots in a 0.5 % NaOCl solution (Hussey and Baker, 1973). The eggs released from the roots were collected on a 25-µm pore size sieve, counted on three 0.025 ml subsamples, and used as inoculum for the test. Egg concentration was adjusted to contain 1,000 and 500 eggs suspended in 2-ml of water.

Two different experiments were conducted. In the first experiment, conducted in 2012, we measured the root galling caused by *M. incognita* feeding in G- and P-type plants,

and in *B. juncea*, *B. oleracea*, and *S. lycopersicum*. The latter three plant species were used as controls. Among these, *B. juncea* and *B. oleracea* have been shown to be less susceptible to *M. incognita* than *S. lycopersicum* (Curto et al., 2005; Edwards and Ploeg, 2014). A total of five plants of each type were used to test differences in root galling. *Barbarea vulgaris* and *B. oleracea* plants were three months old at the beginning of the experiment in June 2012. *Brassica juncea* plants were three weeks old and *S. lycopersicum* plants were six weeks old at the beginning of the experiment. Plants of the different types tested were randomly transplanted into a greenhouse-covered field with naturally occurring *M. incognita* nematodes. The minimum distance between adjacent plants was 50 cm. Plants were uprooted three months later and root galling was estimated in the laboratory using a scale from 0 to 10 (0 = no galls, 5 = 50% galled) (Bridge and Page, 1980). This galling index was used as a measure of host-plant resistance to *M. incognita*, as other studies have used similar galling indexes to measure host-plant resistance to this and other plant parasitic *Meloidogyne* spp. (Mukhtar et al., 2017; Zhang and Schmitt, 1994). In the second experiment, conducted in 2017, only G- and P-type plants were tested and two densities of nematode inoculum were used, either 500 or 1.000 eggs of *M. incognita* suspended in 2 ml of water. To infest the plants, two 3-cm deep holes were made on opposite sides of the plant, approximately 2 cm away from the plant, and 1 ml of the suspension of nematode inoculum was added to each of the two holes, which were subsequently covered with soil. A total of 5-7 plants were used for each plant type and density of nematode inoculum. After 10 weeks, plants were uprooted and roots were examined in order to assess root-galling and to calculate the number of nematode eggs found in the roots and the multiplication rate (number of eggs at the end of the experiment/number of eggs inoculated at the beginning of the experiment)

per plant. This multiplication rate was used to assess host-plant suitability for the nematode, as other studies have used similar multiplication rates and reproduction factors to assess host-plant suitability in *Meloidogyne* spp. (Mukhtar et al., 2017; Zhang and Schmitt, 1994).

2.3 Resistance and susceptibility to western flower thrips

This experiment was conducted to test differences in *F. occidentalis* thrips densities and leaf damage in G- and P-type plants. The experiment relied on the naturally-occurring *F. occidentalis* thrips in the greenhouse where the experiment was conducted. Plants of the two types were randomly located on a bench at the greenhouse and the minimum distance between adjacent plants was 15 cm. Plants were 8 weeks old when the observations were made in October 2011 and the experiment included a total of 20 plants of each type. The observations recorded included number of immature and adult *F. occidentalis* per plant and whether symptoms of leaf damage by *F. occidentalis* were visible with the naked eye. The symptoms of feeding by *F. occidentalis* thrips were visible as leaf scars and silvery patches containing greenish-black fecal specks. The experiment was replicated in November 2011 using 10 plants of each type.

2.4 Statistical analysis

Data comparing the percentage of G- and P-type plants showing symptoms of infection by the mildew *E. cruciferarum* and damage by the thrips *F. occidentalis* were analysed using a one-tailed, two-sample test of proportions with STATA® version 14.2 (StataCorp, 2015). For G- and P-type plants, data comparing the percentage of leaves per

plant showing symptoms of infection by the mildew *E. cruciferarum*, leaf diameter, total number of leaves, and differences in *E. cruciferarum* infection according to leaf diameter were analyzed using the Mann-Whitney U Test with SPSS® version 24 (IBM, 2017). To compare the susceptibility/resistance of the different host plants to the nematode *M. incognita*, data comparing root galling, number of eggs, and multiplying factor were also analyzed using the Mann-Whitney U Test with SPSS®. To compare the densities of *F. occidentalis* thrips on plants, data were analyzed using the Kruskal-Wallist Test with with SPSS®.

3. Results

3.1 Resistance and susceptibility to powdery mildew

For the first replication of the experiment, the percentage of plants showing symptoms of *E. cruciferarum* mildew infection was 20.8 and 62.5% for G- and P-type plants, respectively (Fig. 1). G-type plants had significantly higher number of leaves, but smaller leaf diameter compared to P-type plants ($P \leq 0.05$) (Table 1). For both G- and P-type plants, leaves with larger diameter were more likely to be affected by *E. cruciferarum* ($P \leq 0.05$) (Table 1). In the second and third replications of the experiment 83.3 and 54.2% of the P-type plants showed symptoms of infection by *E. cruciferarum*, while none of the G-type plants showed symptoms of infection by the pathogen. Differences in the percentage of plants showing symptoms of infection by *E. cruciferarum* in G- and P-type plants were statistically significant for the first ($z = 2.9$; $P = 0.002$), second ($z = 5.9$; $P \leq 0.001$) and third ($z = 4.2$; $P \leq 0.001$) replications of the experiment (Table 1). The percentage of leaves affected by *E. cruciferarum* per plant was also significantly higher in

P-type than in G-type plants ($P \leq 0.001$) for all the three replications of the experiment (Table 1). *Barbarea verna* and *B. rupicola* plants did not show any symptoms of infection by *E. cruciferarum*.

3.2 Resistance and susceptibility to root-knot nematode

In the first experiment there were no significant differences in root galling between G- and P-type plants ($P > 0.05$) (Fig. 2). There were also no significant differences in root galling between G-type and *B. oleracea* ($P > 0.05$) and between P-type and *B. oleracea* ($P > 0.05$). Root galling was significantly lower in *B. juncea* than in G- and P-type plants ($P \leq 0.05$). Root galling was significantly higher in *S. lycopersicum* than in G- and P-type plants ($P \leq 0.05$). In the second experiment, there were no significant differences in root galling between G- and P-type plants ($P > 0.05$) (Fig. 3). There were also no significant differences between G- and P-type plants in the number of eggs per root fresh weight found at the end of the experiment ($P > 0.05$) (Fig. 4). The multiplication rate of the nematode *M. incognita* was, however, significantly higher in P-type than in G-type plants in the treatment with the higher level of initial infestation (1,000 eggs/plant) ($P \leq 0.05$) (Fig. 5). In the treatment with the lower level of initial infestation (500 eggs/plant) there were no significant differences in the multiplication rate of *M. incognita* between the two types. The higher level of initial infestation with *M. incognita* eggs resulted in significantly higher values of root galling, eggs per root, and multiplication rate than the lower level of initial infestation ($P \leq 0.05$).

3.3 Resistance and susceptibility to western flower thrips

In the first replication of the experiment, the percentage of leaves per plant showing symptoms of damage by the thrips *F. occidentalis* was 2.1 and 88.3% for G- and P-type plants, respectively, and this difference was statistically significant ($z = 5.5$; $P < 0.001$) (Fig. 6). In the second replication of the experiment, 2.9 and 73.1% of leaves per plant showed symptoms of damage by *F. occidentalis* in G- and P-type plants, respectively, and this difference was statistically significant ($z = 3.3$; $P = 0.001$). The total numbers of immature *F. occidentalis* found on P-type and G-type plants were, respectively, 32.8 and 0.2 per plant in the first replication, and 24.2 and 0.2 on the second replication ($P \leq 0.05$) (Fig. 7A). The total number of adult *F. occidentalis* found on P-type and G-type plants were, respectively, 2.9 and 0.6 per plant in the first replication, and 2.4 and 0.1 on the second replication ($P \leq 0.05$) (Fig. 7B).

4. Discussion

This study shows that the G-type is significantly less susceptible to damage by the mildew *E. cruciferarum* and the thrips *F. occidentalis* than the P-type.

There were no significant differences between the G- and P-types in the root galling caused by the nematode *M. incognita* in the experiments conducted in a greenhouse-covered field, with maximum temperatures reaching 43 ºC, and in a growth chamber at 24 ºC. Lack of significant differences in root galling indicated similar levels of resistance to the nematode in the G- and P-types. However, one of the laboratory experiments showed that the multiplication rate of the nematode was higher on the P-type than on the G-type in the higher level of infestation. The higher multiplication rate of the nematode on the P-type indicates that it is a better host for this nematode than the G-type in this particular

experiment, and possibly in general. Nematode reproduction and root-galling are not always coupled in root-knot nematode-host plant interactions (Liébanas and Castillo, 2004; Roberts et al., 2008; Zhang and Schmitt, 1994). For example, broccoli cv. Calabrese and waterchestnut had the same galling index when attacked by the nematode *Meloidogyne konaensis*, however, on waterchestnut the reproduction factor of the nematode was 87 times bigger than on broccoli cv. Calabrese (Zhang and Schmitt, 1994).

Glucosinolates and saponins have been shown to provide defense against herbivorous insects, nematodes, and fungal pathogens (Bowyer et al., 1995; Christian and Hadwiger, 1989; De Geyter et al., 2012; Halkier and Gershenzon, 2006; Hopkins et al., 2009; Osbourn, 1996; Singh and Kaur, 2018). Given the differences in glucosinolate and saponin content between G- and P-type plants (Agerbirk et al., 2015; Christensen et al., 2014), the low susceptibility to these pests could be due to differences in these plant defense metabolites. Since the mildew *E. cruciferarum* occurred mainly in the leaves of larger diameter in *B. vulgaris*, which are known to contain lower glucosinolate and saponin content than leaves of smaller diameter within the same plant (Badenes-Pérez et al., 2014a), this indicates that glucosinolates and/or saponins could be associated with resistance to this pest. Like most of the G-type plants used in the study, *B. rupicola* and *B. verna* plants were also not affected by the mildew. *Barbarea rupicola* and *B. verna* also contain some saponins, but in lower concentrations than the G-type (Badenes-Pérez et al., 2014b). *Barbarea verna* and an unknown type of *B. vulgaris*, possibly G-type or similar, have also been shown to contain antifungal phytoalexins (Pedras et al., 2015). The content of these phytoalexins in P-type plants is not known, but these metabolities could also be responsible for the difference resistance to the mildew. The amount and composition of leaf cuticular

302 wax has been shown to affect plant susceptibility to *E. cruciferarum* (Weis et al., 2014).
303 The apparent glossiness of the G-type and *B. verna* seems to indicate that these plant types
304 contain lower amounts of wax on the leaf surface than the P-type (Badenes-Pérez, personal
305 observation). The differences in resistance and susceptibility to the ascomycete *E.*
306 *cruciferarum* in the G- and P-types is opposite in the case of the oomycete *A. candida*, for
307 which the G-type is the most susceptible type (Christensen et al., 2014; Heimes et al., 2014;
308 van Mölken et al., 2014). The case of resistance to the rust *A. candida* is the only one in
309 which resistance to a herbivore is detected in the P-type rather than in the G-type, which
310 besides showing resistance to the mildew *E. cruciferarum* and the thrips *F. occidentalis* as
311 we report here, is already well-known as resistant to *P. xylostella* and *P. nemorum*
312 (Agerbirk et al., 2003b; Badenes-Pérez et al., 2014b; Idris and Grafius, 1994).

313 Although the thrips *F. occidentalis* has been shown to exhibit strong host plant
314 preferences (Cao et al., 2014; Nyasani et al., 2013), it is not clear how the preference is
315 determined between the G- and the P-types. A different thrips species, onion thrips, *Thrips*
316 *tabaci* Lindeman (Thysanoptera: Thripidae), which also feeds on cruciferous crops (Nault
317 et al., 2014; Smith et al., 2011), was also found on *B. vulgaris* May through October in
318 Elba, NY, US (Smith et al., 2011). Although the type and variety of *B. vulgaris* were not
319 specified in the study by Smith et al. (2011), the *B. vulgaris* plants reported in Ithaca, NY,
320 US (approximately 190 km away from Elba) were G-type var. *arcuata* (Agerbirk et al.,
321 2003b; Badenes-Pérez et al., 2004). There are no known reports on the existence of P-type
322 plants in the US. In cabbage, leaf wax content seems to provide resistance against *T. tabaci*
323 and a negative correlation between UV-A and visible reflection of leaves and antixenotic
324 resistance has been shown (Bálint et al., 2013; Trdan et al., 2008). In several plant species

outside the family Brassicaceae it has been shown that waxes can increase reflectance of both UV and visible wavelengths, while pubescence can significantly increase reflection at visible wavelengths (Holmes and Keiller, 2002). Since the G-type appears to be less waxy than the P-type, rather than an antixenotic resistance to the thrips *F. occidentalis* due to leaf reflectance, an antibiotic resistance due to saponins could more likely, but this remains to be tested.

This study indicates that the G-type could be a source of genes of resistance to the mildew *E. cruciferarum* and the thrips *F. occidentalis*, especially considering that reports on plant resistance to these herbivores are scarce (Bradshaw et al., 1989; Jones et al., 2013; Reitz, 2009; Sasser, 1980; Singh et al., 2010; Uloth et al., 2016; Van de Wouw et al., 2016). In the case of *F. occidentalis*, we did not find any report of constitutive resistance in Brassicaceae and we only found one case of induced resistance (Abe et al., 2009). When cross-resistance to several herbivores occurs, selection for increased resistance to one herbivore can also enhance resistance to the other herbivore species for which the plant is resistant (Leimu and Koricheva, 2006). This makes the G-type very attractive to facilitate plant breeding for multiple pests. Since the first identification of a feeding-deterrent saponin in *B. vulgaris* (Shinoda et al., 2002), several *Barbarea* spp. have been suggested as a source of feeding-deterrents for *P. xylostella* and *P. nemorum*, and as sources of genes for selection and development of plant varieties resistant to these insect pests (Agerbirk et al., 2003a; Badenes-Pérez et al., 2014b; Kuzina et al., 2009; Nielsen et al., 2010b). Although key genes responsible for the biosynthesis of the saponin 3-*O*-*β*-cellobiosylhederagenin still remains to be identified, QTL mapping, together with the recent sequencing of the genome of the G-type, and re-sequence of the P-type, is narrowing

348 the genomic region containing QTLs for saponin production and insect resistance (Byrne
349 et al., 2017; Khakimov et al., 2015; Kuzina et al., 2011).
350
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565
566

Table 1. Percentage of leaves infected with the powdery mildew *Erysiphe cruciferarum*, total number of leaves, and leaf diameter, including the diameter of leaves with and without mildew infection (means ± SE) in G- and P-type plants.

	G-type	P-type	*P*-value
Percentage of plants with mildew infection[a]	6.9 ± 6.9	66.5 ± 8.6	$P \leq 0.005$
Percentage of leaves with mildew infection[a, b]	5.1 ± 0.8	23.5 ± 1.1	$P \leq 0.001$
Number of leaves[c]	40.2 ± 3.2	23.2 ± 2.9	$P \leq 0.05$
Leaf diameter (cm)[c]	2.7 ± 0.1	3.6 ± 0.2	$P \leq 0.05$
Leaf diameter (cm) of leaves with mildew infection[c]	4.4 ± 0.1	5.1± 0.1	$P \leq 0.05$
Leaf diameter (cm) of leaves without mildew infection[c]	2.6 ± 0.1	3.1 ± 0.2	$P \leq 0.05$

[a]: Data as the average from the three replications of the experiment. Difference statistically significant with a one-tailed, two-sample test of proportions at $P \leq 0.05$.

[b]: Considering only the plants that showed visible mildew infection symptoms.

[c]: Data from the first replication of the experiment in which these measurements were taken. Difference statistically significant with the Mann-Whitney U Test at $P \leq 0.05$.

Figure Captions:

Fig. 1. Percentage of leaves with symptoms of infection by the mildew *E. cruciferarum* per plant (mean + SE) in G- and P-type plants. Observations were made in experiments conducted in December 2014, September 2015, and December 2015.

Fig. 2. Root galling index (mean + SE) as a result of root feeding by the nematode *M. incognita* in plants of G- and P-type *B. vulgaris*, *B. juncea*, *B. oleracea*, and *S. lycopersicum*. Root galling was estimated on a scale from 0 to 10 (0 = no galls, 5 = 50% galled) as in Bridge and Page (1980).

Fig. 3. Root galling index (mean + SE) as a result of root feeding by the nematode *M. incognita* in G- and P-type plants under initial infestation levels of 500 and 1,000 *M. incognita* eggs/plant. Root galling was estimated on a scale from 0 to 10 (0 = no galls, 5 = 50% galled) as in Bridge and Page (1980).

Fig. 4. Number of eggs of the nematode *M. incognita* per fresh weight of root plant (mean + SE) in G- and P-type plants. Initial infestation levels were 500 and 1,000 *M. incognita* eggs/plant.

Fig. 5. Multiplication rate of the nematode *M. incognita* calculated as final number of eggs/initial number of eggs inoculated (mean + SE) in G- and P-type plants. Initial infestation levels were 500 and 1,000 *M. incognita* eggs /plant.

Fig. 6. Percentage of leaves per plant with symptoms of feeding damage by the thrips *F. occidentalis* (mean + SE) in G- and P-type plants. Observations were made in October (n = 20) and November 2011 (n = 10).

Fig. 7. Number of immature (A) and adult (B) *F. occidentalis* thrips per plant (mean + SE) in G- and P-type plants. Observations were made in October (n = 20) and November 2011 (n = 10).

Fig. 1

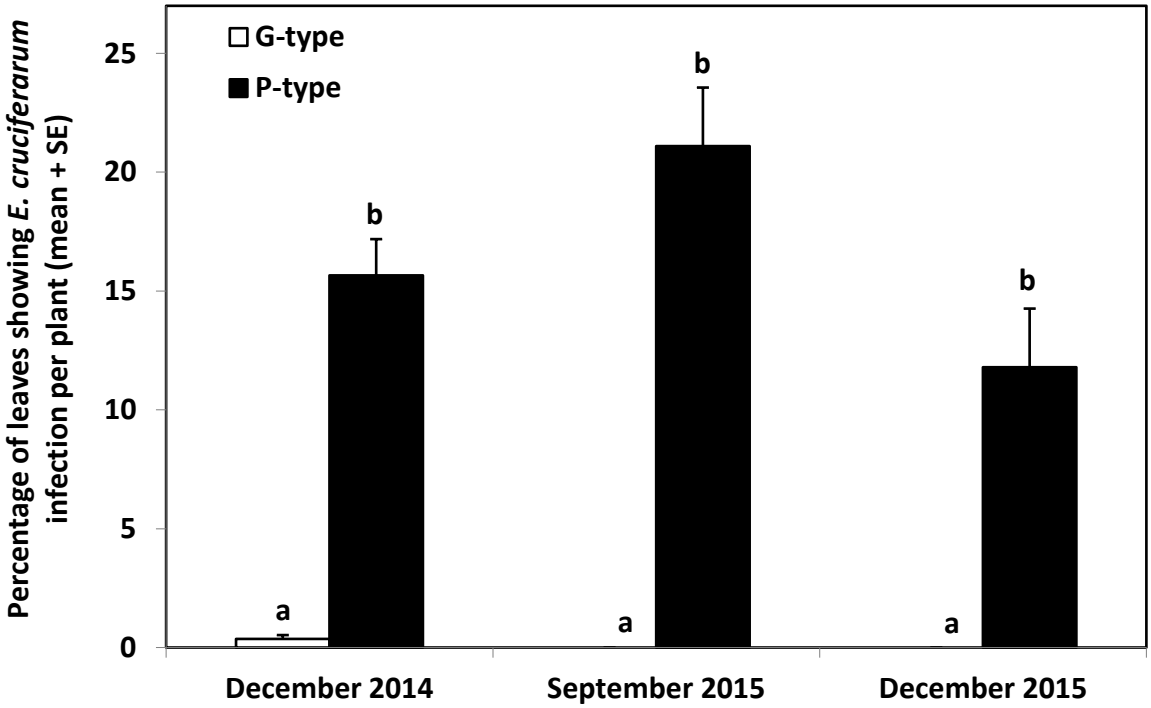

G-type
P-type
Percentage of leaves showing E. cruciferarum infection per plant (mean + SE)
25
20
15
10
5
0
a
b
a
b
a
b
December 2014
September 2015
December 2015

Fig. 2

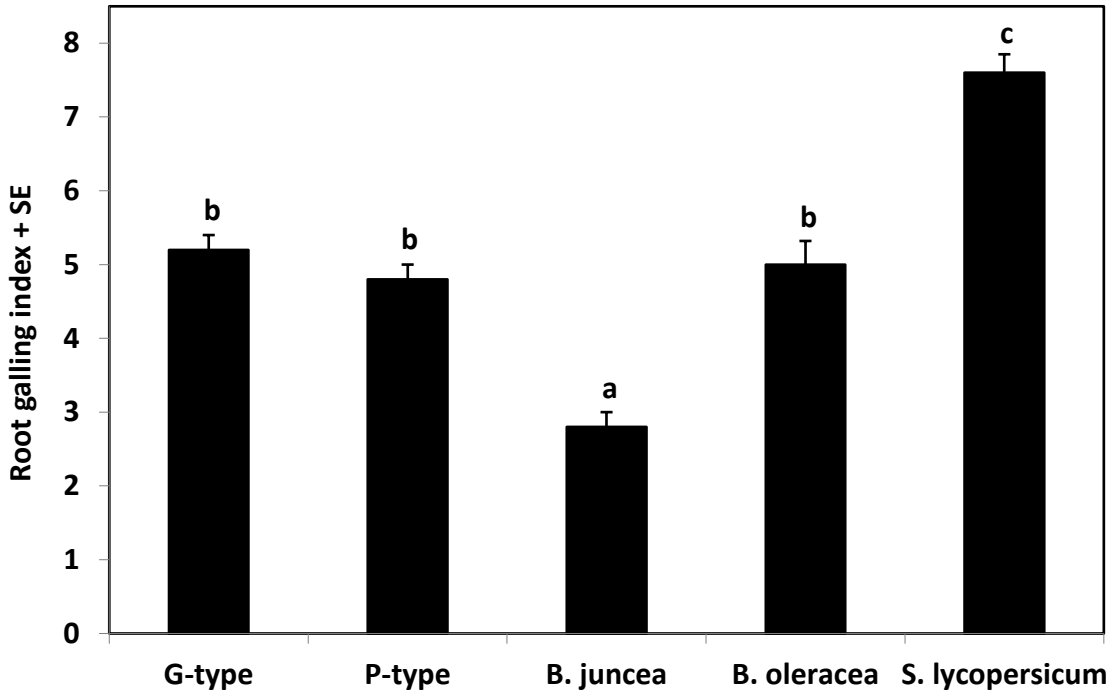

Root galling index + SE
0
1
2
3
4
5
6
7
8
b
b
a
b
c
G-type
P-type
B. juncea
B. oleracea
S. lycopersicum

Fig. 3

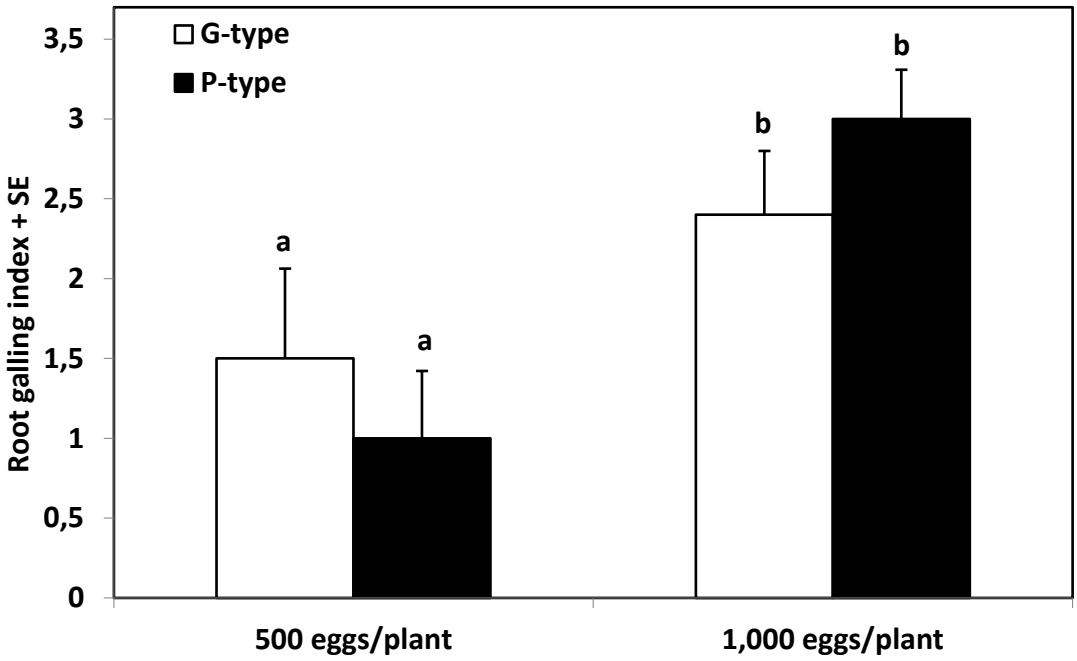

G-type
P-type
Root galling index + SE
3,5
3
2,5
2
1,5
1
0,5
0
a
a
b
b
500 eggs/plant
1,000 eggs/plant

Fig. 4

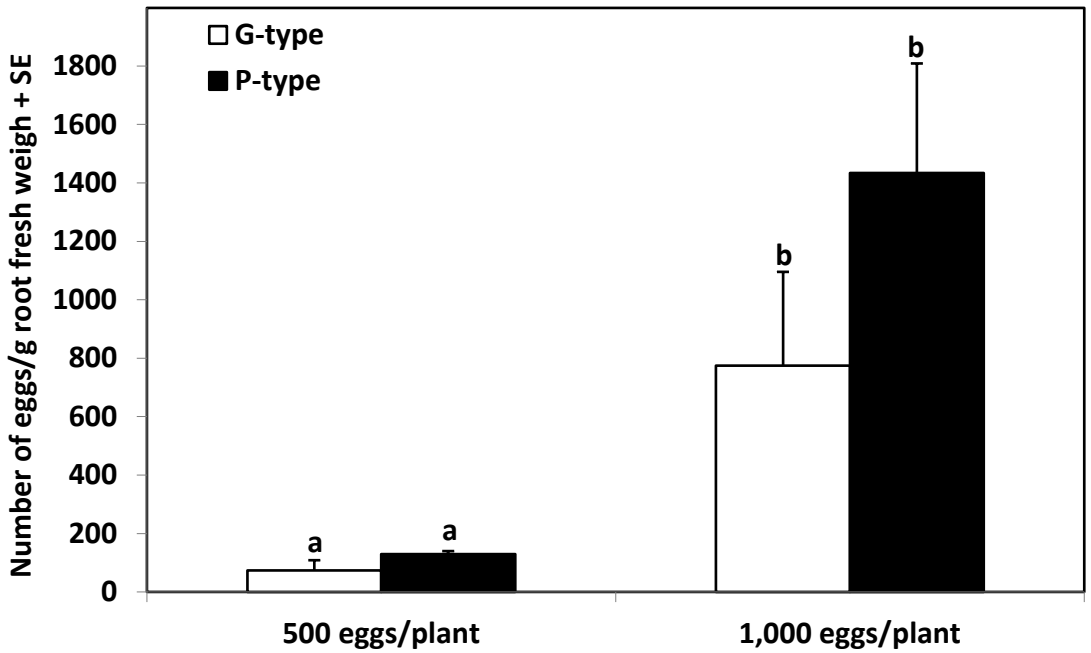

G-type
P-type
Number of eggs/g root fresh weigh + SE
1800
1600
1400
1200
1000
800
600
400
200
0
a
a
b
b
500 eggs/plant
1,000 eggs/plant

Fig. 5


G-type
P-type
Multiplication rate + SE
0
1
2
3
4
5
6
7
a
a
a
b
500 eggs/plant
1,000 eggs/plant

Fig. 6

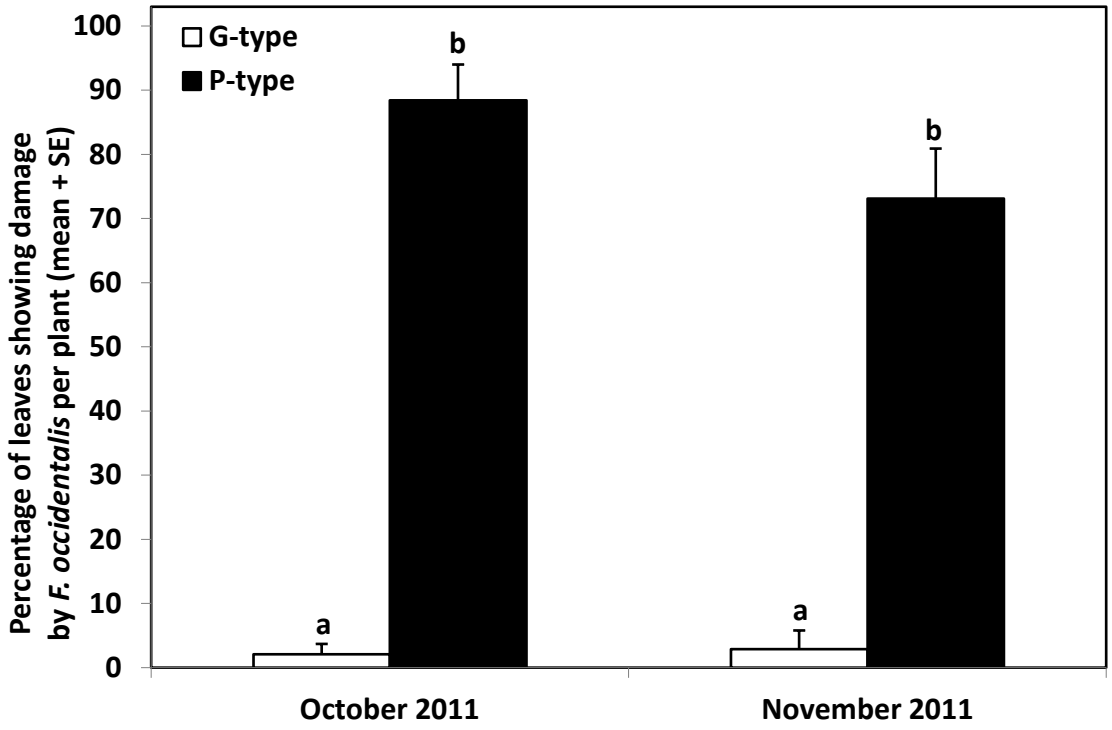
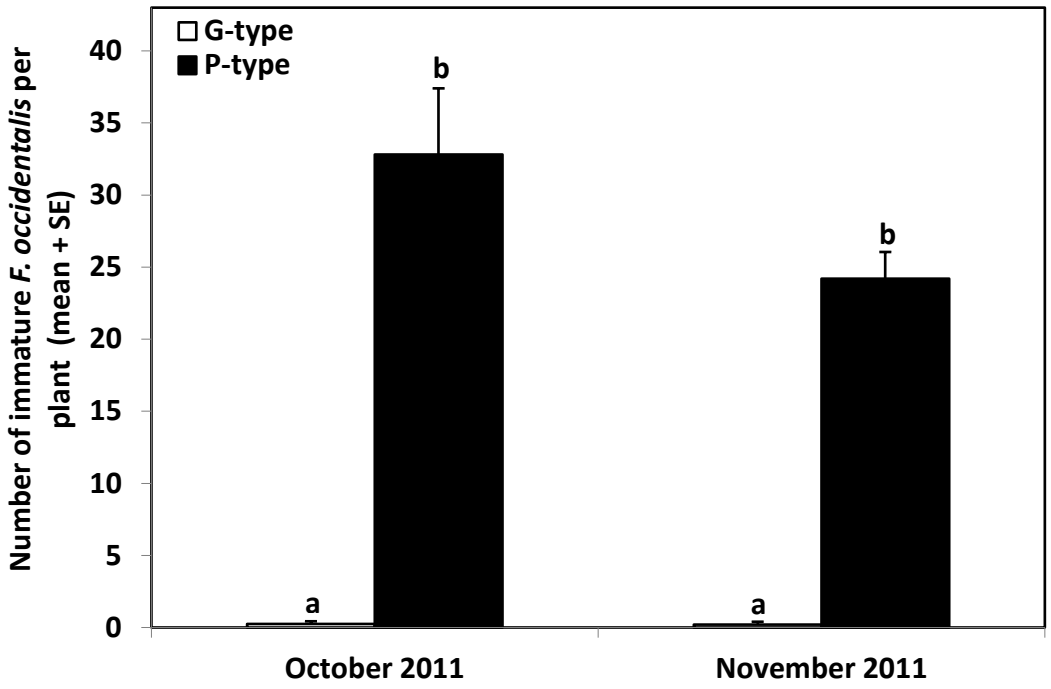
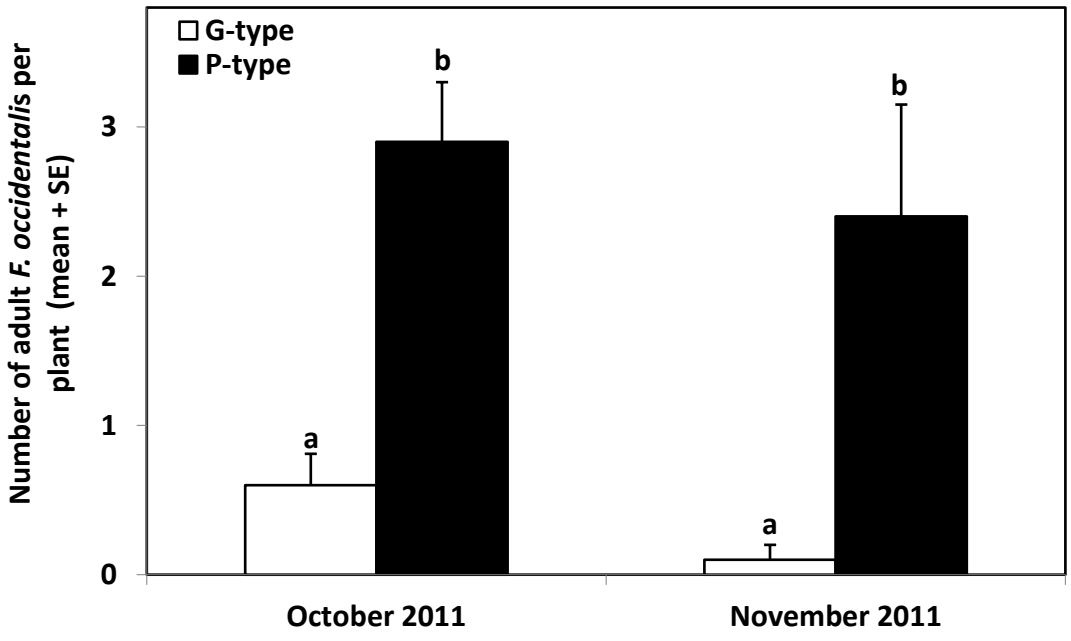

G-type
P-type
Percentage of leaves showing damage by *F. occidentalis* per plant (mean + SE)
100
90
80
70
60
50
40
30
20
10
0
a
b
a
b
October 2011
November 2011

Fig. 7

A


G-type
P-type
Number of immature F. occidentalis per plant (mean + SE)
40
35
30
25
20
15
10
5
0
a
b
a
b
October 2011
November 2011

B


G-type
P-type
Number of adult F. occidentalis per plant (mean + SE)
3
2
1
0
a
b
a
b
October 2011
November 2011